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Etiology of Acute Undifferentiated Febrile Illness in the Amazon Basin of Ecuador

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Abstract. We conducted a longitudinal observational study of 533 patients presenting to two hospitals in the Ecuadorean Amazon basin with acute undifferentiated febrile illness (AUFI) from 2001 through 2004. Viral isolation, reverse transcription-polymerase chain reaction (RT-PCR), IgM seroconversion, and malaria smears identified pathogens responsible for fever in 122 (40.1%) of 304 patients who provided both acute and convalescent blood samples. Leptospirosis was found in 40 (13.2%), malaria in 38 (12.5%), rickettsioses in 18 (5.9%), dengue fever in 16 (5.3%), Q fever in 15 (4.9%), brucellosis in 4 (1.3%), Ilhéus infection in 3 (1.0%), and Venezuelan equine encephalitis (VEE), Oropouche, and St. Louis encephalitis virus infections in less than 1% of these patients. Viral isolation and RT-PCR on another 229 participants who provided only acute samples identified 3 cases of dengue fever, 2 of VEE, and 1 of Ilhéus. None of these pathogens, except for malaria, had previously been detected in the study area.

INTRODUCTION

Although acute undifferentiated febrile illness (AUFI) is common in tropical regions of the developing world, its specific etiology is often unknown, making accurate diagnosis, effective treatment, and targeted public health measures difficult. With no focal signs or symptoms and a scarcity of available diagnostic tests, health care providers in these underserved areas have great difficulty identifying a specific pathogen, with the potential for increased morbidity and mortality.[1–8] Previous studies have found leptospirosis, malaria, rickettsioses, and arboviral diseases such as dengue fever, Venezuelan equine encephalitis (VEE), and Oropouche to be among the prevailing causes of AUFI in the tropical regions of Peru, Brazil, and Venezuela.[3,4,8–14] To date there are no published data from Ecuador. The current research sought to identify pathogens causing AUFI in the Amazon basin of Ecuador, and specific disease characteristics that might assist clinicians in distinguishing between various etiologies.

METHODS AND MATERIALS

Study site. Study participants were recruited from April 2001 through September 2004 at two hospitals in the Pastaza province of the Amazon basin of Ecuador. Hospital Vozandes del Oriente is a mission hospital located in the town of Shell that treats patients of diverse ethnic and socioeconomic backgrounds from throughout the region. Hospital de la IV División de Amazonas is a military hospital in the provincial capital of Puyo that predominantly attends to Ecuadorean Army personnel and their dependents, many of whom are stationed at remote jungle outposts.

Eligibility criteria. Patients were eligible for enrollment in the study if they presented to either hospital with a documented oral temperature of at least 38°C for 7 days or less accompanied by one or more of the following signs and symptoms: headache, myalgia, ocular pain, abdominal pain, arthralgia, generalized fatigue, cough, nausea or vomiting, sore throat, rhinorrhea, dyspnea, diarrhea, hematochezia, jaundice, dizziness, disorientation, stiff neck, rash, petechiae, ecchymosis, epistaxis, or gingival bleeding. Patients < 5 years of age were excluded unless they displayed hemorrhagic manifestations. Also excluded were individuals with a readily identifiable focus of infection, such as otitis media, sinusitis, purulent pharyngitis, cellulitis, urinary tract infection, dental abscess, septic arthritis, pneumonia, pelvic inflammatory disease, or peritonitis.

Ethical considerations. Signed informed consent was obtained from all study participants. Patients < 18 years of age were required to give verbal consent to be included in the study, after which a written consent form was signed by a parent or legal guardian. Participants were recruited from the outpatient clinic, emergency department, and inpatient wards of both hospitals, with the goal of including all patients that met the enrollment criteria. Less than 5% of eligible patients declined to participate in the study. This study was approved by the research ethics committee of Hospital Vozandes del Oriente, the Pastaza provincial director of the Ecuadorean Ministry of Public Health, and the U.S. Naval Medical Research Center Institutional Review Board (Protocol NMRCD.2001.0002) in compliance with all applicable federal regulations governing the protection of human subjects.

Data collection. Epidemiologic and clinical data including signs and symptoms were recorded on standardized forms. A complete blood count, malaria smear, and serum collection were performed on acute blood samples taken at the time of enrollment. Participants were requested to return for a free medical exam 14 to 28 days later, at which time a convalescent serum sample was collected. Sera were stored at −70°C until they could be shipped on dry ice to the U.S. Naval Medical Research Center Detachment (NMCRD) in Lima, Peru.

Viral isolation. Acute-phase sera were diluted 1:5 in minimum essential medium containing 2% heat-inactivated fetal bovine serum and antibiotics. African Green Monkey Vero and mosquito C6/36 cell cultures were each inoculated with 200 μL of the diluted serum in 25 mL flasks. Upon observation of viral cytopathic effect (CPE), or 10 days post-

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14. ABSTRACT

We conducted a longitudinal observational study of 533 patients presenting to two hospitals in the Ecuadorean Amazon basin with acute undifferentiated febrile illness (AUFI) from 2001 through 2004. Viral isolation, reverse transcription-polymerase chain reaction (RT-PCR), IgM seroconversion, and malaria smears identified pathogens responsible for fever in 122 (40.1%) of 304 patients who provided both acute and convalescent blood samples. Leptospirosis was found in 40 (13.2%), malaria in 38 (12.5%), rickettsioses in 18 (5.9%), dengue fever in 16 (5.3%), Q fever in 15 (4.9%) brucellosis in 4 (1.3%), Ilh? infection in 3 (1.0%), and Venezuelan equine encephalitis (VEE), Oropouche, and St. Louis encephalitis virus infections in less than 1% of these patients. Viral isolation and RT-PCR on another 229 participants who provided only acute samples identified 3 cases of dengue fever, 2 of VEE, and 1 of Ilh?. None of these pathogens, except for malaria, had previously been detected in the study area.

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inoculation if no CPE was observed, cells were removed from the flasks and placed on 12-well glass spot-slides for examination by immunofluorescence assay (IFA) using dengue-1, dengue-2, dengue-3, dengue-4, yellow fever, St. Louis encephalitis, Ilhéus, VEE, Oropouche, and arthropod-borne group C (which includes viruses such as Apeu, Caraparu, Itaqui, Marituba, Murutucu, and Oriboca) virus-specific antibodies. For a subset of patient samples, if a time-sensitive response was called for, acute-phase serum samples were tested for the presence of pathogen-specific nucleotide sequences by reverse transcription-polymerase chain reaction (RT-PCR). Dengue sequences were first amplified using pan-dengue primers followed by serotype-specific nested primers.[15]

Serology. Pathogen-specific IgM titers were determined by using an IgM-capture enzyme-linked immunosorbant assay (ELISA). Briefly, plates (96-well format) were coated with anti-human IgM antibody to capture patient IgM molecules. Virus-specific IgM was detected by the addition of viral antigen, followed by virus-specific hyperimmune ascitic fluid and HRP-labeled anti-mouse IgG. Following the addition of colorimetric substrate, plates were read at 490nm. All acute and convalescent samples from the participants who provided both specimens were initially screened at 1:100. Samples exceeding the reference cut-off value, calculated as the median of seven antibody-negative samples plus three standard deviations, were considered IgM-positive. Positive samples were subsequently re-tested at 4-fold serial dilutions to determine end-point titers.

A 4-fold increase in titer from acute to convalescent sample, or a PanBio test which went from negative to positive, was considered indicative of seroconversion. Additionally, samples in which the acute IgM was negative and the convalescent IgM was positive were also considered to be positive for acute infection.

Viruses included in serologic diagnoses were dengue-1, dengue-2, dengue-3, dengue-4, yellow fever, St. Louis encephalitis, Ilhéus, VEE, Oropouche, Mayaro, and arthropod-borne group C viruses. New tests were added as they became available, so only the samples were tested for Ilhéus, Saint Louis encephalitis, and group C arboviruses. Paired samples that were negative for these viral infections were serologically tested for *Coxiella burneti*, *Leptospira*, *Rickettsiae*, and *Brucella* using PanBio kits. Serology was performed only for acute and convalescent serum specimens from patients for which the viral isolation was negative. Malaria was detected based solely on a single acute later blood smear. Figure 1 shows the laboratory screening process.

Data analysis. Survey, clinical, and laboratory data were entered into a spreadsheet using Excel and Epi Info 3.3.2 (Centers for Disease Control and Prevention, Atlanta, GA). SPSS 16.0 (SPSS, Chicago, IL) was used for statistical analysis, and the significance level was set at $\alpha = 0.05$.

RESULTS

Study population. Of the 533 participants who enrolled in the study, 304 (57.0%) returned to provide a convalescent blood sample and 229 (43.0%) provided only an acute specimen. Two-sided χ^2 tests were used to compare those who returned for a follow-up visit to those who did not. The mission hospital had a higher rate of return (64.9%) than the military hospital (46.2%, $P < 0.001$). The return rate was higher for children ages 0 to 17 (65.7%) and adults ages 45 years and higher (63.2%) than for adults ages 18 to 44 (50.0%, $P = 0.002$). There was no statistically significant difference for the rate of return for males (54.7%) and females (61.8%, $P = 0.135$). Of the 304 individuals who provided both acute and convalescent specimens, 200 (65.8%) were enrolled at the mission hospital and 104 (34.2%) at the military hospital. There were 107 (35.2%) females and 197 (64.8%) males, and the mean age was 22.6 years (SD, 14.6 years). Participants included 136 (44.7%) children age 0 to 17, 59 (19.4%) young adults age 18 to 24, 85 (28.0%) adults age 25 to 44, and 24 (7.9%) adults age 45 and higher. The military hospital had a higher proportion of male participants (86.5%) than the mission hospital (53.5%, $P < 0.001$) and also had a higher mean age (26.4 years) than the mission hospital (18.8 years, $P < 0.001$).

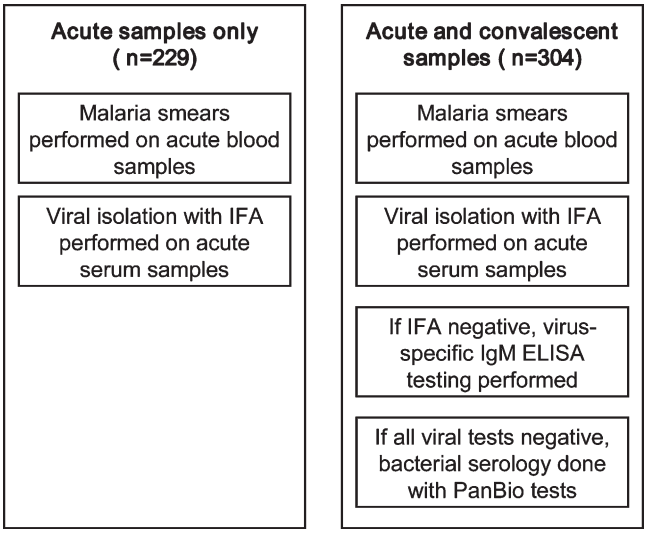

FIGURE 1. Laboratory flow-chart.

Laboratory findings. Diagnosis was made by isolation of a pathogen from the acute specimen using IFA, RT-PCR, or IgM seroconversion in the cases in which both acute and convalescent specimens were available, or by thick and thin smears in the case of malaria, as described in the Methods section and as shown in Figure 1. Results for the 304 participants with both acute and convalescent specimens are shown in Table 1 and discussed in the next five paragraphs (results for the 229 patients with acute samples only are provided at the end of this section). All 304 participants were tested for five agents (dengue, yellow fever, Oropouche, Mayaro, and VEE) and fewer were tested for the remaining agents, as noted on Table 1. At least one bacterial, viral, or parasitic pathogen was detected in 122 (40.1%) of these 304 participants. Nineteen (15.6%) of these 122 patients were positive for two pathogens and 4 (3.3%) for three pathogens. These findings are consistent with multiple infections or cross-reactive serologic assays.

Leptospirosis and malaria were the most commonly diagnosed infections in the study. Forty cases of leptospirosis were found out of the 272 patients tested by seroconversion. Leptospirosis was more common in males (32/197) than females (8/107, $P = 0.033$). Thirty-eight of 298 patients tested for malaria were infected. The species was determined for 32 cases; 29 had *Plasmodium vivax* infection and 3 had *Plasmodium falciparum* infection. Sixteen cases of dengue fever were detected, including 14 diagnosed by seroconver-

TABLE 1

Fever-causing pathogens identified by serologic conversion of IgM, viral isolation, reverse transcription-polymerase chain reaction (RT-PCR) and/or malaria blood smear for the 304 participants who provided both acute and convalescent samples

Infectious agent	Number positive	Number negative	Number not tested	% Positive of tested	% Positive of all 304
Dengue	16	288	0	5.3	5.3
Yellow fever	10	294	0	3.3	3.3
Oropouche	1	303	0	0.3	0.3
Venezuelan equine encephalitis	1	303	0	0.3	0.3
Mayaro	0	304	0	0.0	0.0
Leptospira	40	212	32	14.7	13.2
Malaria	38	260	6	12.8	12.5
Coxiella	15	18	54	6.0	4.9
Rickettsia typhi	8	247	49	3.1	2.6
Rickettsia rickettsii	6	208	86	2.8	2.0
Rickettsia prowazekii	5	253	41	1.9	1.6
Brucella	4	271	27	1.3	1.3
Ilhéus	3	8	293	27.3	1.0
St. Louis encephalitis	1	10	293	9.1	0.3
Group C arboviruses	0	31	273	0.0	0.0

sion, 9 by IFA (of which 8 were also detected by seroconversion), and 1 by RT-PCR. A serotype was determined for 10 of the dengue cases (9 by IFA and 1 by RT-PCR): 5 were DEN1, 3 were DEN2, and 2 were DEN3. Dengue fever was more common in adults (14/168, 8.3%) than children (3/136, 2.2%, $P = 0.017$). Cutaneous eruptions were more common in dengue patients (4/16, 25.0%) than participants without dengue (10/288, 3.5%, $P < 0.001$). There were no cases of dengue hemorrhagic fever.

Fifteen patients with acute and convalescent specimens tested positive for *Coxiella burneti* and 19 for *Rickettsiae*, all by seroconversion. *Brucella* was identified by seroconversion in 4 participants, Oropouche was detected by both seroconversion and IFA in 1 patient, and 1 case of VEE was found by seroconversion. Ilhéus was found in 3 and St. Louis encephalitis in 1 of the 11 samples tested for seroconversion to these pathogens.

Yellow fever IgM seroconversion was found in 10 adults. Two hundred twenty-three participants reported having previously received a yellow fever vaccine, including all 10 with IgM seroconversion. Five of these 10 patients reported a date for their most recent yellow fever vaccination, and all the dates were within 5 years before study enrollment.

Except as noted previously, there were no statistically significant differences in diagnosis by age group, sex, or clinical presentation. The most common signs and symptoms for the 304 participants (in addition to fever) were headache (82.2%), myalgia (65.1%), nausea (47.0%), abdominal pain (41.1%), eye pain (28.0%), vomiting (27.3%), cough (26.3%), throat pain (25.7%), eye infection (18.1%), coryza (11.2%), lymphadenopathy (5.3%), cutaneous eruptions (4.6%), hepatomegaly (4.3%), jaundice (3.3%), and bleeding (3.3%).

Pathogens were also identified by IFA and/or RT-PCR in 6 of the 229 additional participants who provided only an acute sample: 3 had dengue (1 DEN1 by IFA, 1 DEN3 by IFA, and 1 DEN3 by RT-PCR), 2 had VEE (1 by IFA and 1 by both RT-PCR and IFA), and 1 had Ilhéus (by IFA).

DISCUSSION

The purpose of this study was to identify the various etiologies and clinical presentations of acute undifferentiated febrile illness in the Amazon basin of Ecuador, with the goal of assisting local health professionals in diagnosing, treating, and preventing these diseases. Fever-causing pathogens were identified in 40% of patients who provided acute and convalescent samples, with leptospirosis and malaria infections predominating. A lesser proportion of patients were found to be acutely infected with *Rickettsiae*, dengue virus, and *Coxiella*. Testing for Ilhéus[16] and St. Louis encephalitis viruses became available late in the study. Although these pathogens were detected in less than 1% of participants overall, 27.3% of the 11 patients tested were positive for Ilhéus and 9.1% were positive for St. Louis encephalitis. Because no yellow fever outbreak was documented or reported during the study period, IgM seroconversion in our 10 previously vaccinated patients was most likely because of a heterologous flavivirus that was not tested for, rather than actual yellow fever infection. It is important to note that none of the identified pathogens except malaria and yellow fever had previously been detected in the study area; hence, their presence was unknown to local clinicians and public health authorities.

Our results are not unlike those found in other tropical regions of the developing world, although the relative incidence of specific pathogens varies considerably. Leptospirosis, malaria, scrub typhus, murine typhus, *Rickettsia typhi*, and dengue have been identified as major causes of AUFI in Thailand, Malaysia, and Nepal.[6,17–22] Dengue was found to cause one-third of all cases of acute undifferentiated non-malarial fever in an area of Vietnam.[5] In South America, spotted fever group *Rickettsia*, leptospirosis, and *Coxiella burneti* have been identified as major identifiable causes of AUFI in a subtropical area of northwestern Peru.[9] Dengue, VEE, malaria, and Oropouche were found in AUFI patients in the Amazon basin of Peru.[14] VEE virus has been identified as the cause of large epidemics of acute, self-limited febrile disease in Venezuela and Colombia.[23]

The difficulty of local health care providers establishing a specific etiology in cases of AUFI in remote areas of the tropics cannot be overemphasized. Accurate diagnosis is complicated by a lack of knowledge of the scope of local pathogens, absence of etiology-specific signs and symptoms, and unavailability of accurate diagnostic testing, particularly during the early phase of illness. For instance, a study in a dengue-endemic area of Vietnam demonstrated the inability of physicians to accurately diagnose the disease, regardless of their

level of training or years of clinical experience.[5] This difficulty is not just the result of a lack of access to advanced laboratory equipment; clinicians in the developed world likewise struggle to find a specific etiology for AUFI acquired in the tropics. In a study by Freedman and others,[24] the cause of systemic febrile illness could not be identified by physicians at academic travel medicine clinics in more than half of travelers returning to the United States from South America. In another series, no etiology could be determined in at least one-fourth of hospitalized febrile travelers returning to the United Kingdom from the tropics.[25]

Given their similar clinical presentations, many researchers have commented on the difficulty in distinguishing between dengue fever and leptospirosis.[3,4,7] In places where dengue is recognized as a significant health problem, leptospirosis may be overlooked as the cause of AUFI, delaying antibiotic administration and leading to increased complications and death.[1,26] During concurrent epidemics in a Brazilian city, leptospirosis initially misdiagnosed as dengue led to a delay in referral for specialized care and an increased risk of intensive care unit (ICU) admission and death.[3] A post-mortem study of suspected dengue patients in Puerto Rico showed that 83% had actually had leptospirosis.[2] Likewise, VEE and dengue can be difficult to distinguish. Serum randomly drawn from Venezuelans of all ages during a supposed VEE outbreak revealed IgG against VEE in 55.2% and 41.9% against dengue, suggesting that the epidemic was likely caused by both pathogens.[8]

Even when dengue fever and leptospirosis are suspected, currently available rapid serologic tests cannot reliably detect IgM antibodies until at least the sixth or seventh day of clinical illness.[27–30] Rapid serologic testing was only able to identify half the cases of dengue and leptospirosis during an outbreak in Thailand.[6] Consequently, during the acute illness clinicians are forced to rely on their knowledge of local pathogens and empirically treat for possible common etiologies.[9,17–19]

Coxiella was identified as the cause of AUFI in nearly 5% of our study participants in Ecuador. Q fever has been recognized as an emerging cause of acute febrile illness in Spain, France, the United Kingdom, Israel, French Guyana, and Mali, with more severe cases commonly manifesting pneumonia and/or hepatitis.[31–42] Although frequently undiagnosed, most patients with Q fever do well, particularly those who receive prompt antibiotic therapy.[31,35,36,39,42] Mortality has been seen in a number of patients with Q fever complicated by myocarditis or meningoencephalitis, and a high incidence of spontaneous abortion, fetal loss, and low birthweight.[38]

Although previously undetected in the Amazon basin of Ecuador, this research identified leptospirosis as a major cause of AUFI in the study area. Leptospirosis outbreaks have been reported following periods of heavy rainfall in urban areas of Brazil, Barbados, and Thailand.[4,6] Leptospirosis IgM or high titers of leptospiral antibodies were found in half the cases of AUFI in Iquitos, a large city in the Amazon region of Peru.[13] Leptospirosis seroprevalence was found to be 28% in an Iquitos slum, with seroconversion being common between the dry and wet seasons.[43] An elegant study by Ganoza and others[44] showed a higher concentration of pathogenic *Leptospira* in the Iquitos water supply compared with nearby rural water sources. This may explain the observation that more severe cases of leptospirosis occur in urban areas.[13]

Although expected, it was disappointing that few clinical factors could be statistically correlated with specific etiologies in the current study. Rash was more commonly seen in dengue than with the other pathogens identified. A case series in Venezuela identified dengue as the most common cause of acute febrile syndrome with rash; IgM for dengue was found in 40.6% of such patients.[10] Dengue was also more common in adults than children, a finding that is difficult to explain. Leptospirosis and malaria were more often found among soldiers in our study, likely a result of increased exposure to these pathogens while living at jungle outposts. Military personnel stationed in the tropics have previously been identified to be at increased risk for leptospirosis.[12]

As an initial effort to determine some of the etiologies of AUFI, the current study has a number of limitations. There is a risk of selection bias given that significantly fewer young adults, and particularly participants from the military hospital, returned for their second visit and were therefore excluded from serologic testing. Many potential pathogens (perhaps most notably the enteric fevers) were not assessed, and samples collected later in the study were subjected to a broader battery of serologic testing. Malaria may have been underdiagnosed, because logistical constraints only allowed for a single malaria smear to be performed on acute blood samples. Finally, because serology for bacterial pathogens was only performed on samples that were negative for viral serology, it is possible that bacterial infections were also underdiagnosed, especially since co-infections were documented in other patients.

Notwithstanding these limitations, this study reports for the first time a number of important pathogens that have been overlooked in the Amazon region of Ecuador. These results have been disseminated to the local medical community and public health authorities, and have already made a significant impact. The discovery of dengue prompted the provincial ministry of health to launch a major vector control effort. Local clinical laboratories now offer serologic testing for dengue and leptospirosis. Such testing is admittedly of little utility early in the course of AUFI, but can be useful to establish the etiology during outbreaks and for patients who present after several days of illness. Local clinicians have also altered their approach to the treatment of AUFI. Typically this now involves ruling out malaria with a blood smear, then treating adults empirically for possible leptospirosis, Q fever, or other Rickettsial diseases with doxycycline (a macrolide antibiotic is substituted for children) and acetaminophen. If typhoid is in the differential diagnosis, ciprofloxacin or a third-generation cephalosporin is added to the treatment regimen. Until simple, affordable tests become available to accurately determine the etiology of AUFI early in its course, a combination of epidemiologic surveillance, focused public health efforts, and broad-spectrum empiric therapy will have to suffice.

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